An Empirical Test of the F_2 Screen for Detection of *Bacillus thuringiensis*-Resistance Alleles in Tobacco Budworm (Lepidoptera: Noctuidae)

CARLOS A. BLANCO,[1,2] O. P. PERERA,[1] FRED GOULD,[3] DOUGLAS V. SUMERFORD,[4] GERARDO HERNÁNDEZ,[5] CRAIG A. ABEL,[1] AND DAVID A. ANDOW[6]

J. Econ. Entomol. 101(4): 1406–1414 (2008)

ABSTRACT Insects exposed to genetically modified crops expressing *Bacillus thuringiensis* (Bt) toxins are under intense selection pressure that could result on widespread Bt resistance. Screening for early indications of Bt resistance developing in targeted Lepidoptera is conducted in many of the regions where genetically modified cotton and corn have been commercialized. *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) has been selected in the laboratory to have a gene for resistance to Cry1Ac. We used this laboratory line to test the assumptions and theoretical predictions related to detection of recessive Bt-resistant alleles in field populations based on a second generation (F_2) screen. By creating single-pair families from mating a heterozygous Cry1Ac-resistant moth with a Cry1Ac-susceptible moth, we simulated the most common genotype when Bt-resistance alleles are at low frequency in the field. The second generation (F_2) neonates of single-pair families were screened daily with diagnostic concentration bioassays. Cry1Ac-resistant homozygous larvae were detected, but the proportion of resistant larvae was generally below the theoretical expectation of 6.25% and was influenced by the moth F_1 sib-mating density and by the day of oviposition of F_2 eggs. Logistical considerations such as F_1 sib-mating density and F_2 neonate screening are important for the successful implementation of a reliable method.

KEY WORDS *Heliothis virescens*, insecticide resistance management, Cry1Ac, YHD2, single-pair family

Insecticide resistance is a world-wide phenomenon with potential for great economic burden and environmental consequences due to loss of pest control and impacts of higher use of insecticides. Plans for the mitigation or delay of insecticide resistance are based on two general components: the "chemical component" that includes rotation of insecticides, restriction of certain ingredients to specific times, and/or the replacement of certain insecticides by others of better performance, etc.; and the "biological component" that relies on the use of pest-tolerant or self-protected crop varieties, planting dates, quarantines, and/or the dilution of insecticide resistant genes from the pest genetic pool by the genetic exchange between resistant and susceptible individuals, among other tactics. An example of the efficient use of the biological component is the resistance management strategy envisioned for transgenic crops (Roush 1997a,b; Andow and Hutchinson 1998; Gould and Tabashnik 1998; Gould 1998; Matten and Reynolds 2003), that has been used to maintain the effectiveness of genetically modified crops for the past 10 yr.

To produce accurate information about the presence/absence of early stages of resistance evolution, a reliable method that can detect resistant alleles in field populations is essential. When insecticide resistant alleles are rare in field populations and are recessive, a second generation (F_2) screen method is necessary to identify them because insects with resistant alleles that are collected from the field are expected to be heterozygous and therefore susceptible to the insecticide. Isolines derived from females and/or males can concentrate resistant alleles into homozygous F_2 offspring that can be distinguished by discriminating concentrations of insecticide (Andow and Alstad 1998).

The tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), is an important pest in North America that has developed resistance to many classes of insecticides (Sparks 1981, Luttrell et al. 1987,

Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by The U.S. Department of Agriculture or by any of the universities participating in this study.

[1] USDA–Agricultural Research Service, Southern Insect Management Research Unit, Stoneville, MS 38776.

[2] Corresponding author, e-mail: cblanco@ars.usda.gov.

[3] Department of Entomology, North Carolina State University, Raleigh, NC.

[4] USDA–Agricultural Research Service, Corn Insects and Crop Genetics Research Unit, Genetics laboratory, Iowa State University, Ames, IA 50011.

[5] Departamento de Matemáticas, Universidad Autónoma Metropolitana-Ixtapalapa, on sabbatical leave from CINVESTAV, Mexico, D.F.

[6] University of Minnesota, St. Paul, MN 55108.

Table 1. Response of various *H. virescens* crosses and colonies to the *B. thuringiensis* Cry1Ac protein, experiment 1

Treatment (generation)	*n*	Slope ± SE	Significance of slope		IC_{50} (μg/ml diet)[a]		Goodness of fit	
			χ^2	Prob.	Dose	95% FL	χ^2	Prob.
1. YHD2♀ × YHD2♂	80	0.711 ± 0.136	27.37	<0.0001	3,358	2,230–6,076	6.59	0.15
2. ARS♀ × ARS♂ (P_0)	80	1.108 ± 0.146	57.20	<0.0001	0.153	0.11–0.19	0.59	0.98
3. YHD2♀ × ARS♂ (P_0)	80	1.213 ± 0.131	84.74	<0.0001	4.947	4.31–5.57	4.74	0.44
4. ARS♀ × YHD2♂ (P_0)	80	1.104 ± 0.172	41.13	<0.0001	1.474	0.75–2.10	10.2	0.07
5. YHD2-ARS♀ × ARS♂ (F_1)	80	1.583 ± 0.180	77.36	<0.0001	0.370	0.30–0.42	2.41	0.78
6. ARS♀ × YHD2-ARS♂ (F_1)	80	1.579 ± 0.171	84.70	<0.0001	0.398	0.33–0.45	5.57	0.34
7. ARS-YHD2♀ × ARS♂ (F_1)	80	1.873 ± 0.201	86.56	<0.0001	0.433	0.37–0.48	4.47	0.48
8. ARS♀ × ARS-YHD2♂ (F_1)	80	1.938 ± 0.204	90.00	<0.0001	0.435	0.38–0.48	4.87	0.43

[a] Inhibitory concentration that arrested the development of all larvae beyond second instar.

Hardee et al. 2001, Terán-Vargas et al. 2005), and it has a low *Bacillus thuringiensis* (Berliner) (Bt) Cry1Ac toxin resistance frequency (Gould et al. 1997). This pest is one of the main targets of genetically modified cotton (*Gossypium hirsutum* L.) that expresses *B. thuringiensis* proteins (Bt cotton).

There are several F_2 screening method that have been implemented for a few insect species such as *Ostrinia nubilalis* (Hübner) (Andow et al. 1998), *Diatraea saccharalis* (F.) (Huang et al. 2007) and *Helicoverpa armigera* (Hübner) (Mahon et al. 2007). Here, we evaluated the feasibility of developing an accurate F_2 screen for *H. virescens* based on the Andow and Alstad (1998) model by using a known *B. thuringiensis*-resistant strain with a specific mutation in a cadherin-like gene (YHD2 colony, Gould et al. 1995). A heterozygous Bt-resistant YHD2 moth was crossed with a homozygous Bt-susceptible moth and sib-mating of their F_1 offspring gave rise to F_2 larvae that were tested for Bt resistance. The fact that only one parent carried a single copy of the mutant allele makes this empirical approach more realistic because field-collected moths that have Bt resistance alleles are most likely to be heterozygotes in the early stages of resistance evolution. This method will be also applicable when screening a homozygous Bt-resistant insect.

The development of an accurate method such as an F_2 screen and its logistical considerations for a practical implementation would allow measuring shifts on the Bt resistance allelic frequency over time and provides evidence about the effectiveness of theoretically based screening approaches.

Materials and Methods

Tobacco Budworm Colonies. Two distinct *H. virescens* strains were used in this study. The first is a colony that is highly resistant to Cry1Ac protein (yhd2), and it is maintained at the North Carolina State University (Gould et al. 1995). This colony is homozygous for a mutation in a cadherin-like gene that confers Bt resistance (Gahan et al. 2001). Although the resistance to Bt in this strain is due to more than a single gene, most of the resistance is contributed by the mutation in the cadherin-like gene (Heckel et al. 1997). The second strain is a Cry1Ac homozygous susceptible reference colony (ARS) maintained at the USDA–Agricultural Research Service facility in Stoneville, MS (Blanco et al. 2005).

Crosses. We produced eight types of neonates (treatments) that were created by the following crosses: yhd2 moths (treatment 1) were pair-mated with ARS moths (treatment 2) to produce heterozygous neonates (yhd2♀-ARS♂ [treatment 3] or ARS♀-yhd2♂ [treatment 4]). The resulting heterozygous parental "P_0" moths were pair-mated with ARS moths (Diagram 1, step II) in four crosses: 1) yhd2-ARS♀ pair-mated (×) with an ARS♂ to produce (treatment 5) 2 ARS♀ × yhd2-ARS♂ (treatment 6), 3) ARS-yhd2♀ × ARS♂ (treatment 7), and 4) ARS♀ × ARS-yhd2♂ (treatment 8). These four crosses produced four types of F_1 single-pair families (diagram 1, step III). These single-pair F_1 families were sib-mated to produce the F_2 generation (diagram 1, step IV).

Moths were held in 500-ml plastic containers (model 42505LY, Consolidated Plastic Co., Twinsburg, OH) covering the top with cloth (Batist, Zweigart, Piscataway, NJ), given free access to 10% sucrose solution, and maintained in an incubator at 27 ± 0.4°C, 75 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Eggs laid on cloth were placed in a freezer sandwich bag (94600, Ziploc, Crawfordsville, IN), and larvae were reared on insect artificial diet (modified from Shaver and Raulston 1971) under the previously described environmental conditions.

Experiment 1 Cry1Ac-Ausceptibility Bioassays. To effectively differentiate yhd2 homozygous larvae from heterozygous and ARS homozygous larvae, a Cry1Ac discriminating concentration was obtained from different bioassays. Neonates obtained on the second and third oviposition day from treatments 1–8 (Table 1) were exposed to a series of dilutions of Cry1Ac (MVP II insecticide, Mycogen Corporation, San Diego, CA) incorporated into the previously referred insect artificial diet to determine the growth inhibitory concentrations for neonates (Siegfried et al. 2000). Eight Cry1Ac concentrations (ranging from 0.063 to 5,000 μg of Cry1Ac/ml of diet, depending on the cross) were tested. Each concentration of a particular tobacco budworm cross was replicated five times with 1.0 ± 0.15 ml of diet dispensed into 16 wells of a bioassay tray (BAW-128, C-D International, Pitman, NJ) (Blanco et al. 2007a). After larval placement on diet, wells were covered with self-adhesive mem-

branes (BIO-CV-16, C-D International) then stored under the previously described environmental conditions for seven days. In addition to observable dead larvae, individuals that survived but had not molted to third instar after 7 d were considered also in the "mortality" category (Siegfried et al. 2000). Probit analyzes were performed using Proc Probit Log Normal from SAS program version 9.1 (SAS Institute 2001) considering nonoverlapping intervals to be significantly different.

Experiment 2 Segregation and Detection of Cry1Ac-Resistant Genotypes. This experiment was aimed to determine the appropriate density of F_1 adults in a mating container to ensure adequate adult survival and per capita fecundity. In addition, we wanted to test the assumption that resistance would be inherited in the F_2 generation consistent with predicted Mendelian ratios. There may be inadvertent selection for or against resistance alleles when F_1 individuals are chosen to be in the mating container, or there may be viability selection during F_1 development, sexual selection during sib-mating, or fecundity selection in the F_1 adults that could distort Mendelian ratios away from the predicted 0.0625.

We chose equal-aged F_1 sibs from each of the four P_0 crosses (diagram 1, step III) to initiate F_1 sib-mating. Because they were F_1 sibs, we did not know the genotype of the individuals, although we expected the Mendelian ratio of one resistant heterozygote to one wild-type homozygote. Sibs were chosen to have a 1:1 sex ratio with total moth densities of 8, 16, and 24 in 500-ml containers under the previously described environmental conditions. These moth densities were chosen to maximize the use of space while not adversely affecting their reproductive biology. Before moth enclosure, half of the virgin males (≤24 h old) from each treatment were given free access to rhodamine B (Sigma-Aldrich, St. Louis, MO) diluted in 10% sucrose solution ("treated" males) for 2 d. The rest of the ≤24-h-old virgin males ("untreated") and all the ≤ 24-h-old virgin females had free access to only 10% sucrose solution for 2 d. Rhodamine intake by half of the males allowed differentiation between rhodamine-treated and untreated spermatophores inside the females, providing a measure for estimating random mating among moths (Blanco et al. 2006a). Males and females were then combined and allowed to mass-mate within full-sib families. This experiment was repeated four times at two different dates for a total of eight replications. In half of the replicates, rhodamine-treated males were also marked on a front wing with a marker pen (Sharpie Fine Point, Bellwood, IL), whereas on the other half of the replications, untreated males were marked on the front wing with a marker pen. These markings enabled us to determine if rhodamine intake had an effect on male mortality. The mortality of F_1 moths was recorded daily for four consecutive days.

F_2 generation eggs (diagram 1, step IV) were collected and recorded daily and placed in freezer sandwich bags for four consecutive days (4 "oviposition days"). Neonates were allowed to hatch under the previously described conditions. The number of treated versus untreated spermatophores inside the females was counted by dissecting females at the end of each replication. F_2 neonates of each F_1 single-pair family from each oviposition day were placed in up to 96 wells (depending on availability of sufficient numbers of larvae) containing 10.0 μg of Cry1Ac/ml of the previously referred insect artificial diet and ≤32 F_2 neonates were placed on control (0 μg of Cry1Ac/ml) diet, which has also been dispensed into bioassay trays. Neonates on bioassay trays were maintained under the previously described environmental conditions and evaluated seven days later by recording larval development and mortality, considering "Cry1Ac resistant homozygous" those F_2 larvae that developed to third instar or older. Neonates of treatments 1, 3, and 4 also were tested on 10.0 μg of Cry1Ac/ml to determine genotype segregation. Treatment 2 (ARS♀ × ARS♂) neonates were not tested on 10.0 μg of Cry1Ac/ml due to the fact that this cross does not produce any larvae with the Bt-resistant phenotype.

The experimental design was a split plot where the main unit was treatment. The subunits were the 4 days of egg production and data were analyzed (analysis of variance (ANOVA)) using SAS version 9.1 (SAS Institute 2001). The expected binomial distribution ranges (6.25% according to Mendelian inheritance ratio of 1/16 for isofamilies [treatments 5–8], 25% or one-fourths for heterozygous crosses [treatments 3 and 4] and 100% or 1/1 for yhd2 cross [treatment 1]) for proportions of Cry1Ac resistant homozygous F_2 larvae, adjusted for mortality on control wells the 95% confidence interval about the mean of proportion of Cry1Ac-resistant larvae to see whether data could have corresponded within the expected ranges mentioned above.

Experiment 3 Mating Frequency and Reproductive Parameters of a Known Proportion of "Identified" Heterozygous and ARS Enclosed Moths. An independent study was conducted to determine whether by controlling the actual ratio of heterozygous and homozygous-resistant moths at the moth densities used in experiment 2 would depart from results obtained in this experiment 3. Because in experiment 2 moths were chosen randomly, and their genotype could not be known, therefore a bias toward one genotype could have occurred when the moths were chosen for experiment 2. In this experiment, we controlled genotype ratio to be exactly 1:1, creating synthetic F_1 families (treatments 5i–8i corresponding to treatments 5–8 of the actual F_1 families). We enclosed moths from specifically-maintained strains or crosses at 1:1 sex ratios with four, six, and 12 heterozygous (treatments 3 and 4) and four, six and 12 homozygous (ARS, treatment 2) moths in 500-ml containers as described previously. In two replications, heterozygous males and females were marked on the front wing with a marker pen and males had free access to rhodamine as described previously. On the other two replications of the same composite population, the ARS moths were marked with pen and rhodamine. Egg production and moth mortality were recorded daily for three consec-

utive oviposition days. Marked and unmarked spermatophores inside females, as described in experiment 2 were recorded at the end of each replication. Neonates from each treatment and oviposition day were exposed to 10.0 μg of Cry1Ac/ml and control insect artificial diet in bioassay trays, maintained and evaluated as previously described. The experimental design followed what was described for the segregation and detection of Cry1Ac-resistant genotypes in experiment 2. The experimental design and statistical analysis for experiment 3 follows what was described for experiment 2.

Experiment 4 Assessment of Reproductive Parameters. To assist us in understanding certain reproductive parameters of the different crosses/strains, the number of fertile eggs, moth longevity, and number of spermatophores per female were measured daily. Twenty-one moth pairs of each of the treatments 1–8 and the reciprocal heterozygote matings yhd2-ARS♀ × yhd2-ARS♂ (treatment 9) and ARS-yhd2♀ × ARS-yhd2♂ (treatment 10) were setup as an independent study and held up to 14 d under the previously described conditions. Reproductive parameters were obtained for 14 consecutive days.

The experimental design was a complete randomized design with 21 replications. The subunits were measured over ≤14 d, and data were analyzed by ANOVA for uneven number of replications using SAS version 9.1 (SAS Institute 2001). Least significant difference (LSD) at $P \leq 0.05$ was used to determine significant differences in means between treatments.

Results

Experiment 1. Cry1Ac-susceptibility significantly differed among the different crosses (Table 1). The protein concentration that effectively arrested the development of yhd2 larvae (treatment 1) beyond second instar was in the order of milligrams per milliliter (≥3,000 μg/ml of diet), whereas the concentration delaying the development of the ARS susceptible colony (treatment 2) was 20,000 times lower (≈0.15 μg of Cry1Ac/ml of diet). Significant differences were also found among heterozygous crosses. The offspring from the ARS♀ × yhd2♂ cross (treatment 4) were less Cry1Ac-susceptible than offspring from the yhd2♀ × ARS♂ cross (treatment 3), suggesting a parental effect on susceptibility. Cry1Ac-susceptibility in heterozygous moths crossed with ARS moths that produced the four different types of F_1 isofamilies, was not significantly different among treatments 5–8 (Table 1). From the growth and mortality IC_{50} values obtained from all the crosses, a discriminating concentration of 10.0 μg of Cry1Ac/ml of diet was found to arrest the development of all larvae to second instar or younger, except for the yhd2 × yhd2 offspring resistant genotype, the target of this study. None of the larvae in all the treatments (except treatment 1) developed beyond second instar in concentrations just below 6.6 μg of Cry1Ac/ml of diet.

Experiment 2. Higher moth densities resulted in higher female mortality (Table 2) for both the yhd2-

Table 2. Cumulative mortality (± SE) and reproductive parameters (± SE) of heterozygous (YHD2-ARS + ARS or ARS-YHD2 + ARS) families enclosed at different *H. virescens* moth densities, experiment 2

	Cumulative mortality				Egg production/♀			Spermatophores	
	First day	Second day	Third day	Fourth day	First day	Second day	Third day	Fourth day	Rhodamine	Control
8 moths										
YHD2-ARS♀ + ARS♀	0	12.5 (±3.1)	19.5 (±5.9)	21.8 (±3.1)	28.6 (±5.2)	67.9 (±9.6)	66.9 (±10.5)	68.8 (±9.8)	1.1 (±0.10)	1.2 (±0.10)
YHD2-ARS♂ + ARS♂	9.3 (±4.7)	31.2 (±6.2)	34.3 (±4.1)	34.30						
16 moths										
YHD2-ARS♀ + ARS♀	7.8 (±3.2)	14.5 (±6.5)	24.9 (±9.9)	33.2 (±3.5)	26.4 (±3.4)	65.3 (±8.0)	95.4 (±31.1)	50.4 (±10.4)	0.6 (±0.04)	0.5 (±0.09)
YHD2-ARS♂ + ARS♂	6.2 (±3.2)	12.5 (±4.1)	33.3 (±6.0)	33.30						
24 moths										
YHD2-ARS♀ + ARS♀	2.0 (±1.5)	14.5 (±6.5)	41.5 (±3.4)	49.8 (±3.0)	23.8 (±1.6)	49.6 (±5.6)	54.8 (±9.1)	17.4 (±3.0)	0.6 (±0.06)	0.6 (±0.14)
YHD2-ARS♂ + ARS♂	0.0	27.0 (±6.3)	46.7 (±3.5)	55.0 (±2.2)						
8 mo										
ARS-YHD2♀ + ARS♀	0	12.5 (±4.7)	15.6 (±3.1)	15.60	17.6 (±3.5)	64.0 (±8.7)	39.0 (±8.2)	54.7 (±7.5)	0.6 (±1.0)	0.6 (±1.8)
ARS-YHD2♂ + ARS♀	15.6 (±6.5)	28.1 (±4.7)	31.2 (±3.1)	31.20						
16 moths										
ARS-YHD2♀ + ARS♀	4.1 (±1.0)	22.8 (±3.6)	47.8 (±5.8)	56.1 (±4.1)	14.5 (±3.7)	62.4 (±7.1)	61.0 (±8.4)	68.4 (±10.3)	0.7 (±0.20)	0.6 (±0.20)
ARS-YHD2♂ + ARS♀	10.4 (±3.2)	47.9 (±5.6)	60.4 (±3.1)	60.40						
24 moths										
ARS-YHD2♀ + ARS♀	2.0 (±1.3)	5.1 (±1.5)	39.4 (±7.0)	47.7 (±3.4)	23.1 (±3.7)	53.9 (±5.0)	49.9 (±7.8)	20.1 (±10.3)	0.5 (±0.10)	0.6 (±0.10)
ARS-YHD2♂ + ARS♀	4.1 (±1.5)	20.7 (±5.6)	41.5 (±4.4)	49.8 (±2.2)						

Table 3. Percentage (±95% confidence interval) Cry1Ac-resistant (third instar or older) *H. virescens* larvae detected from four different isofamilies, experiment 2

Treatment	% ≥third instars/oviposition day				
	First	Second	Third	Fourth	*
8 moths					
5. YHD2-ARS♀ × ARS♂	0.5 (±1.7)	2.5 (±1.2)	2.0 (±1.4)	0.25 (±1.0)	b
6. ARS♀ × YHD2-ARS♂	0.5 (±1.0)	1.4 (±1.1)	2.3 (±2.1)	0	b
7. ARS-YHD2♀ × ARS♂	0	7.7 (±1.8)	5.7 (±1.8)	5.7 (±1.8)	ab
8. ARS♀ × ARS-YHD2♂	0	8.8 (±3.7)	16.1 (±11.4)	10.4 (±4.4)	a
16 moths					
5. YHD2-ARS♀ × ARS♂	2.5 (±0.7)	5.2 (±1.5)	6.3 (±3.2)	3.5 (±2.0)	b
6. ARS♀ × YHD2-ARS♂	2.0 (±1.2)	4.0 (±2.2)	3.2 (±1.4)	1.5 (±1.5)	b
7. ARS-YHD2♀ × ARS♂	5.2	9.8 (±1.6)	9.8 (±2.7)	8.0 (±3.6)	a
8. ARS♀ × ARS-YHD2♂	0	6.2 (±3.1)	6.2 (±2.6)	6.2 (1.8)	b
24 moths					
5. YHD2-ARS♀ × ARS♂	3.3 (±1.5)	5.0 (±2.2)	6.0 (±1.9)	5.0 (±2.0)	a
6. ARS♀ × YHD2-ARS♂	1.0 (±2.1)	1.6 (±0.8)	3.0 (±1.2)	2.5 (±1.0)	b
7. ARS-YHD2♀ × ARS♂	1.5 (±1.0)	0.5 (±1.6)	3.1 (±1.9)	3.1 (±1.4)	b
8. ARS♀ × ARS-YHD2♂	0	5.1 (±1.0)	3.6 (±1.8)	6.01	ab

* Rows of moth ratios followed by the same letter are not significantly different at $P < 0.05$.

ARS and ARS F_1 family (density $F = 5.61$; df = 2, 10; $P = 0.02$/day $F = 6.00$; df = 3, 11; $P = 0.01$) and the ARS-yhd2 and ARS F_1 family (density $F = 0.0008$; df = 2, 11; $P = 7.72$/day $F = 7.15$; df = 3, 11; $P = 0.0002$). However, density did not affect male mortality for either F_1 family: yhd2-ARS and ARS (density $F = 0.97$, df = 2, 11; $P = 0.40$/day $F = 2.52$; df = 3, 11; $P = 0.11$) and ARS-yhd2 and ARS (density $F = 3.60$; df = 2, 8; $P = 0.07$/day $F = 2.73$; df = 3, 11; $P = 0.09$). No significant differences were found in the average number of eggs produced at different moth densities by either yhd2-ARS or ARS females (density $F = 1.15$; df = 2, 83; $P = 0.32$/day $F = 2.02$; df = 3, 83; $P = 0.11$) or ARS-yhd2 and ARS females (density $F = 0.67$; df = 2, 11; $P = 0.53$,/day $F = 0.42$; df = 3, 11; $P = 1.0$). There were no significant differences in the number of treated (rhodamine) and untreated spermatophores found inside yhd2-ARS and ARS females ($F = 0.21$; df = 4, 43; $P = 0.92$) or inside ARS-yhd2 and ARS females ($F = 0.86$; df = 4, 36; $P = 0.49$) (Table 2).

The percentage of F_2 larvae in the "Cry1Ac resistant" category (third instar or older development) was generally significantly lower than the expected Mendelian inheritance ratio of 6.25%. Of the 43 possible comparisons of 95% confidence intervals, 22 were significantly <6.25% and two were significantly >6.25% (Table 3). When one parent of the initial single-pair family was a heterozygote from a cross of ARS♀-yhd2♂ this proportion was higher compared with the proportion when a parent was yhd2♀-ARS♂ (Table 3). Significant differences in the percentage of resistant larvae were obtained when total moth density was eight ($F = 5.52$, $P = 0.01$, df = 3, 9) with higher numbers of resistant larvae on treatment 8. At a confinement density of 16 moths, there was a significantly higher proportion of resistant larvae detected in treatment 7 ($F = 13.34$; df = 3, 9; $P = 0.001$,). At a confinement density of 24 moths, treatment 5 had a significantly higher proportion of resistant larvae ($F = 4.75$; df = 3, 9; $P = 0.02$). The proportion of Cry1Ac-resistant larvae was closer to the expected 6.25% when neonates of the second and third oviposition days of 16 and 24 moth densities were tested (Table 3). Failure to detect any resistant larvae occurred primarily on the first oviposition day with the highest incidence on the lower moth density.

Experiment 3. Known proportions of "identified" heterozygous and homozygous moth confined at different densities (Table 4) had a significant mortality effect on yhd2-ARS and ARS females (density $F = 5.61$; df = 2, 10; $P = 0.02$/day $F = 6.00$; df = 3, 11; $P = 0.01$) but not on yhd2-ARS and ARS males (density $F = 0.97$; df = 2, 11; $P = 0.40$/day $F = 2.52$; df = 3, 11; $P = 0.11$). Moth density during confinement had a significant mortality effect on ARS-yhd2 and ARS females (density $F = 7.72$; df = 2, 11; $P = 0.0008$/day $F = 7.15$, df = 3, 11; $P = 0.0002$) but not on ARS-yhd2 and ARS males (density $F = 3.80$; df = 2, 11; $P = 0.07$/day $F = 2.73$; df = 3, 11; $P = 0.09$). There were no significant differences in the average number of eggs produced by equal proportions of yhd2-ARS and ARS moths confined at different ratios (density $F = 1.15$; df = 2, 83; $P = 0.32$/day $F = 2.02$; df = 3, 83; $P = 0.11$) or by the eggs produced by ARS-yhd2 and ARS females (density $F = 0.67$; df = 2, 83; $P = 0.53$/day $F = 1.00$; df = 3, 83; $P = 0.42$). There were significant differences in the average number of spermatophores inside yhd2-ARS and ARS females ($F = 3.93$; df = 3, 18; $P = 0.006$), but not in the number of treated or untreated spermatophores ($F = 1.70$; df = 11, 36; $P = 0.11$). There were no significant differences in the average number of spermatophores inside ARS-yhd2 versus ARS females ($F = 1.32$; df = 3, 18; $P = 0.27$), nor in the number of treated or untreated spermatophores ($F = 0.90$; df = 11, 36; $P = 0.54$) (Table 4).

Detection of Cry1Ac-resistant larvae was also slightly lower than expected (6.25%) during confinement of known ratios of identified heterozygous and ARS moths (Table 5). This time no significant differences were found in the average number of Cry1Ac-resistant larvae on mating of both types of identified heterozygous moths (treatment YHD2-ARS or ARS-

Table 4. Cumulative mortality (± SE) and reproductive parameters (± SE) of enclosing a known ratio of "identified" heterozygous (YHD2-ARS or ARS-YHD2) with homozygous (ARS) *H. virescens* moths, experiment 3

	Cumulative mortality				Egg production♀				Spermatophores	
	First day	Second day	Third day	Fourth day	First day	Second day	Third day	Fourth day	Rhodamine	Control
8 "identified" moths										
2 YHD2-ARS♀	0	0	31.2 (±6.2)	31.20	28.6 (±5.2)	67.9 (±9.6)	66.9 (±10.5)	68.8 (±9.8)	1.4 (±0.18)	1.2 (±0.12)
2 ARS♀	0	12.4 (±4.7)	18.8 (±3.3)	18.8					1.5 (±0.13)	1.6 (±0.15)
2 YHD2-ARS♂	18.8 (±2.8)	50.0 (±8.6)	50.00	62.5 (±7.7)						
2 ARS♂	6.3 (±0.9)	18.8 (±4.1)	18.80	44.8 (±8.3)						
16 "identified" moths										
4 YHD2-ARS♀	3.2 (±0.4)	15.7 (±4.1)	53.2 (±6.9)	59.4 (±5.5)	26.4 (±3.4)	65.3 (±8.0)	95.4 (±31.1)	50.4 (±10.4)	1.0 (±0.04)	1.1 (±0.09)
4 ARS♀	6.3 (±1.3)	21.9 (±4.8)	32.8 (±3.9)	39.0 (±2.2)					1.4 (±0.08)	1.4 (±0.06)
4 YHD2-ARS♂	9.4 (±1.1)	25.0 (±2.5)	40.7 (±5.0)	40.70						
4 ARS♂	3.2 (±2.0)	12.5 (±2.9)	25.0 (±6.4)	33.4 (±5.7)						
24 "identified" moths										
6 YHD2-ARS♀	6.3 (±1.5)	18.8 (±5.6)	37.5 (±3.8)	64.4 (±4.5)	23.8 (±1.6)	49.6 (±5.6)	54.8 (±9.1)	17.4 (±3.0)	0.9 (±0.06)	0.8 (±0.15)
6 ARS♀	0.0	12.5 (±3.4)	27.1 (±6.8)	30.2 (±1.4)					1.3 (±0.10)	1.4 (±0.08)
6 YHD2-ARS♂	6.3 (±2.4)	27.1 (±4.3)	41.7 (±6.0)	55.5 (±5.3)						
6 ARS♂	6.3 (±2.1)	27.1 (±3.9)	29.2 (±2.5)	29.20						
8 "identified" moths										
2 ARS-YHD2♀	0	6.3 (±1.1)	12.5 (±3.2)	20.5 (±2.6)	17.6 (±3.5)	64.0 (±8.7)	39.0 (±8.2)	54.7 (±7.5)	0.5 (±0.18)	0.5 (±0.18)
2 ARS♀	0	6.3 (±0.9)	12.5 (±4.1)	30.5 (±6.5)					0.8 (±0.10)	0.8 (±0.12)
2 ARS-YHD2♂	0.0	6.3 (±2.0)	12.5 (±3.9)	19.1 (±1.9)						
2 ARS♂	0.0	6.3 (±1.1)	6.3 (±2.4)	12.5 (±3.3)						
16 "identified" moths										
4 ARS-YHD2♀	3.2 (±0.1)	6.3 (±2.0)	18.8 (±7.3)	25.0 (±4.8)	14.5 (±3.7)	62.4 (±7.1)	61.0 (±8.4)	68.4 (±10.3)	1.2 (±0.20)	1.0 (±0.21)
4 ARS♀	3.2 (±1.1)	9.4 (±3.3)	25.0 (±8.7)	25.0					1.1 (±0.16)	1.0 (±0.20)
4 ARS-YHD2♂	0	15.7 (±3.5)	31.2 (±9.4)	45.0 (±12.5)						
4 ARS♂	0	15.7 (±4.0)	18.8 (±6.3)	37.5 (±9.1)						
24 "identified" moths										
6 ARS-YHD2♀	0	4.2 (±2.6)	25.0 (±5.5)	47.5 (±13.5)	23.1 (±3.7)	53.9 (±5.0)	49.9 (±7.8)	20.1 (±10.3)	0.5 (±0.15)	0.5 (±0.17)
6 ARS♀	0	2.1 (±0.7)	16.7 (±3.5)	25.0 (±8.4)					0.6 (±0.11)	0.4 (±0.09)
6 ARS-YHD2♂	0	2.1 (±1.6)	16.7 (±3.5)	33.5 (±9.2)						
6 ARS♂	0.0	12.5 (±3.6)	29.2 (±13.5)	42.5 (±11.1)						

Table 5. Percent (±95% CI) Cry1Ac-resistant (third instar or older) *H. virescens* larvae from four crosses mated at different ratios of "identified" moths, experiment 3

Treatment	% ≥third instars/oviposition day				*
	First	Second	Third	Fourth	
4 "identified" + 4 ARS moths					
2YHD2-ARS♀ + 2ARS♀ + 2YHD2-ARS♂ + 2ARS♂	1.1 (±1.5)	3.4 (±0.1)	0.9 (±1.3)	1.5 (±1.4)	a
2ARS-YHD2♀ + 2ARS♀ + 2ARS-YHD2♂ + 2ARS♂	1.2 (±0.8)	5.3 (±31.1)	3.9 (±1.7)	2.2 (±3.9)	a
4YHD2♀ + 4YHD2♂	83.2 (±14.1)	82.0 (±18.0)	80.5 (±12.2)	79.6 (±20.4)	
4YHD2-ARS♀ + 4YHD2-ARS♂	6.4 (±5.4)	5.3 (±18.2)	7.7 (±10.9)	3.3 (±3.1)	
4ARS-YHD2♀ + 4ARS-YHD2♂	16.2 (±83.8)	36.0 (±29.6)	21.6 (±29.9)	8.9 (±7.5)	
8 "identified" + 8 ARS moths					
4YHD2-ARS♀ + 4ARS♀ + 4YHD2-ARS♂ + 4ARS♂	0.3 (±0.6)	1.8 (±0.8)	3.4 (±0.1)	2.6 (0.9)	b
4ARS-YHD2♀ + 4ARS♀ + 4ARS-YHD2♂ + 4ARS♂	0.3 (±0.6)	6.4 (±0.8)	6.6 (±1.4)	4.2 (±2.3)	a
8YHD2♀ 8 8YHD2♂	82.0 (±30.5)	85.3 (±14.7)	86.1 (±14.9)	78.5 (±10.3)	
8YHD2-ARS♀ + 8YHD2-ARS♂	8.3 (±37.6)	14.0 (±9.0)	9.7 (±13.2)	1.0 (±13.2)	
8ARS-YHD2♀ + 8ARS-YHD2♂	11.0 (±22.2)	21.0 (±42.2)	15.6 (±39.4)	7.8 (±6.1)	
12 "identified" + 12 ARS moths					
6YHD2-ARS♀ + 6ARS♀ + 6YHD2-ARS♂ + 6ARS♂	3.0 (±1.2)	5.5 (±2.9)	5.8 (±1.3)	9.2 (±2.6)	a
6ARS-YHD2♀ + 6ARS♀ + 6ARS-YHD2♂ + 6ARS♂	1.1 (±1.5)	5.9 (±4.7)	5.5 (±3.2)	4.4 (±12.0)	a
12YHD2♀ + 12YHD2♂	85.0 (±15.0)	86.2 (±13.8)	84.5 (±15.5)	78.8 (±6.7)	
12YHD2-ARS♀ + 12YHD2-ARS♂	16.0 (±23.9)	26.6 (±16.8)	6.3 (±4.1)	1.1 (±1.0)	
12ARS-YHD2♀ + 12ARS-YHD2♂	11.8 (±17.4)	28.4 (±50.0)	10.6 (±16.1)	8.8 (±6.0)	

* Moth ratios in only those two analyzed rows followed by the same letter are not significantly different at $P < 0.05$.

YHD2) confined with homozygous-susceptible moths (treatment ARS-ARS) (eight moths [$F = 4.89$; df = 1, 3; $P = 0.11$], 16 moths [$F = 5.58$; df = 1, 3, $P = 0.09$], and 24 moths [$F = 2.04$, df = 1, 3; $P = 0.24$]). Confinement of heterozygous yhd2-ARS moths produced fewer Cry1Ac-resistant larvae than the expected Mendelian inheritance ratio of 25%, obtaining more third instars or older with matings of treatment 3 at the 16-moth density (eight moths [$F = 7.44$; df = 1, 3; $P = 0.07$], 16 moths [$F = 31.62$; df = 1, 3; $P = 0.01$], and 24 moths [$F = 0.91$; df = 1, 3; $P = 0.40$]). Homozygous yhd2 moths produced significantly fewer Cry1Ac-resistant larvae than the expected 100% ratio, and here it also depended on the confinement ratio and oviposition day (Table 5).

Experiment 4. There were significant differences (Table 6) in the number of fertile eggs ($F = 5.94$; df = 9, 164; $P < 0.0001$), spermatophores per female ($F = 7.33$; df = 9, 190; $P < 0.0001$), and female ($F = 1.98$; df = 9, 159; $P = 0.04$) and male ($F = 3.79$; df = 9, 138; $P = 0.0003$) longevity when different crosses were maintained as pair matings (Table 6), with overall higher values observed in the ARS♀ × ARS♂ and ARS♀ × YHD2-ARS♂ pairs.

Discussion

This work shows that the F_2 screening protocol used here should be effective in segregating and detecting Cry1Ac-resistant tobacco budworms collected from the field. Cry1Ac-resistant larvae can be detected in the second generation of single-pair families created by one Cry1Ac-resistant heterozygous parent. However, important caveats need to be considered (Andow and Alstad 1998): moth density during the F_1 sib-mating and the day when insects are collected (egg days) can influence the reliability of the screening test. Lower densities (≈4♀ and 4♂) of enclosed F_1 moths tended to produce a low or nonexistent proportion of Cry1Ac-resistant larvae during the first and fourth oviposition day. This proportion was still low when known ratios of heterozygous and Cry1Ac-resistant moths were enclosed at the same density (Tables 3 and 5). A possible explanation for the lower-than-expected resistance detection is that heterozygous females lay significantly fewer eggs and the initiation of their oviposition pattern was delayed by almost a day compared with the control strain (Table 6), which means that sampling eggs on the first oviposition day

Table 6. Reproductive parameters of homozygous and heterozygous combination *H. virescens* pairs held for up to 14 d, experiment 4

Treatment	% Fertile females	Total fertile eggs	Spermatophores/female	Longevity (d)	
				Female	Male
1. YHD2♀ × YHD2♂	66	245d	2.92d	10.00ab	11.16ab
2. ARS♀ × ARS♂	90	610a	4.84b	10.77a	11.00ab
3. YHD2♀ × ARS♂	80	314d	3.68cd	8.75bc	7.80c
4. ARS♀ × YHD2♂	71	399bcd	2.58d	7.85bc	8.21c
5. YHD2-ARS♀ × ARS♂	66	358cd	5.00ab	9.07abc	8.45c
6. ARS♀ × YHD2-ARS♂	100	587a	5.75a	8.80bc	11.70a
7. ARS-YHD2♀ × ARS♂	95	552a	4.30bc	8.30bc	8.22c
8. ARS♀ × ARS-YHD2♂	76	498abc	4.31bc	8.00bc	9.00bc
9. YHD2-ARS♀ × YHD2-ARS♂	100	525ab	3.70cd	7.55c	9.28bc
10. ARS-YHD2♀ × ARS-YHD2♂	80	356cd	4.52bc	8.50bc	9.50bc

Means followed by different letters in columns are significantly different at $P < 0.05$.

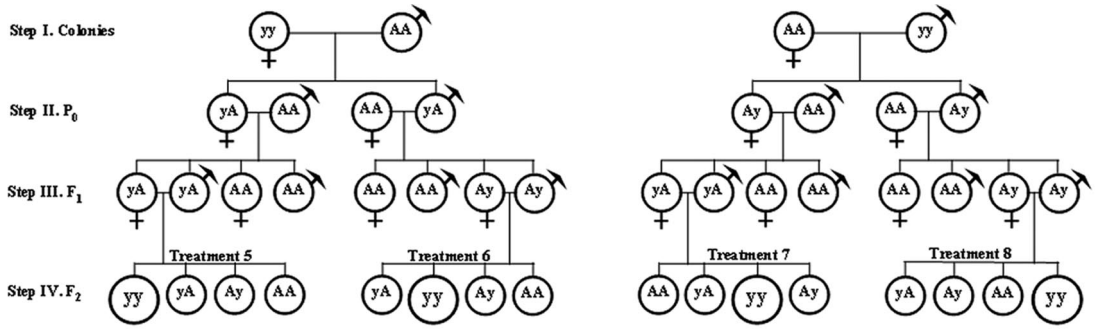

Fig. 1. Theoretical genotypes and sex segregation of the various *H. virescens* crosses.

had a greater chance of "missing" F_2 resistant larvae. When sampling is made during the best oviposition days (second and third) there is a "systematic" under representation of those eggs coming from heterozygous females, according to the reproductive parameters of each cross (Table 6). These reproductive parameters (fertile females and number of fertile eggs) were arithmetically used to predict the "resistant neonate production" of each of the crosses. Results from this synthetic cohort of females also demonstrated that single-pair families with paternal resistant origin (treatments 5 and 6) had a greater hypothetical proportion of resistant larvae (≈7%) than those single-pair families (treatments 7 and 8) with maternal resistant origin (≈4%) (Table 5).

For implementing an F_2 screen for tobacco budworm two factors are critical. First, the best time to obtain eggs for a Cry1Ac screening seems to be on the second and third oviposition day (Tables 3 and 5), and this is also the time when the number of eggs will be more abundant (Tables 2 and 4) (Blanco et al. 2006b). Second, increasing the F_1 sib-mating density from 8 to 16 moths also increased the proportion of Cry1Ac-resistant larvae that were detected, and the reliability of detecting resistant larvae in all of the egg days (Tables 3 and 5). Increasing the F_1 sib-mating densities to 12 females and 12 males could have a deleterious mortality effect noticed when moth reached this density (Tables 2 and 4), but this could be compensated for with larger mating containers (≥500 ml), which in turn would create a greater need for space to hold each single-pair family. Therefore, F_1 sib-mating is recommended at ≈8 females and ≈8 males, despite the high type II error rate associated with these low numbers. Of course the error rate for detecting Cry1Ac-resistant larvae can be reduced by setting-up multiple containers of F_1 sib-matings, but this incur into a greater need of space, time and labor. The recommendation described here represents a reasonable tradeoff between space and resources and detection probability.

The F_2 screen was originally designed to use mated field-caught females (Andow and Alstad 1998). A methodology of mating field-caught males or females with a susceptible moth (laboratory) to create an F_2 family, reduces the number of genes screened in half (Stodola and Andow 2004), but, however, will enhance the chance of obtaining offspring from the heterozygous moth. Our unpublished data indicate that there are 33% more successful pair-copulations when one of the parental moths is laboratory-reared, especially if a female-reared moth is involved. Also, because it is possible to capture males in pheromone traps several weeks before and after when larvae are present in the field, the possibility of using males captured in pheromone traps mated with laboratory reared females expands the opportunity to screen for rare alleles in wild populations by several weeks. This is of particular importance where decreasing numbers of *H. virescens* captured in pheromone traps (Parajulee et al. 2004, Blanco et al. 2005) and larvae in plant hosts (Blanco et al. 2007b), make achieving an adequate sample size difficult.

The results presented here demonstrate that the theoretical (Fig. 1) concept of an F_2 screen (Andow and Alstad 1998), is a valid method for obtaining rare *B. thuringiensis* alleles. This is a labor-intensive method, but it offers reliability for the detection of insecticide-resistant *H. virescens.*

Acknowledgments

We thank Drs. Sharon Downes, YuCheng Zhu, and Debbie Boykin for help in reviewing early drafts of the manuscript and the valuable comments made by two anonymous reviewers. The help of Arnetrius Ellis-Wright, Chad Roberts, and Gloria Paterson on different aspects of this research is greatly appreciated.

References Cited

Andow, D. A., and D. N. Alstad. 1998. F_2 screen for rare resistance alleles. J. Econ. Entomol. 91: 572–578.

Andow, D. A., and W. D. Hutchinson. 1998. Bt-corn resistance management, pp. 19–66. *In* Mellon and J. Rissler [eds.], Now or never: serious new plans to save a natural pest control. Union of Concerned Scientists, Cambridge, MA.

Andow, D. A., D. N. Alstad, Y. H. Pang, P. C. Bolin, and W. D. Hutchinson. 1998. Using an F_2 screen to search for resistant alleles to *Bacillus thuringiensis* toxin in European corn borer (Lepidoptera: Crambidae). J. Econ. Entomol. 91: 579–584.

Blanco, C. A., M. Mullen, C. Abel, J. R. Bradley, P. Ellsworth, J. K. Greene, A. Herbert, R. Leonard, J. D. Lopez, Jr., R. Meagher, et al. 2005. *Bacillus thuringiensis* Cry1Ac resistance monitoring program for the tobacco budworm and bollworm in 2004. *In* Proceedings of the 2005 Beltwide Cotton Conference, 4–7 January 2005, New Orleans, LA. National Cotton Council, Nashville, TN.

Blanco, C. A., O. Perera, J. D. Ray, E. Taliercio, and L. Williams III. 2006a. Incorporation of rhodamine B into male tobacco budworm moths *Heliothis virescens* to use as a marker for mating studies. J. Insect Sci. 6:05 (insectscience.org/6.05).

Blanco, C. A., D. Sumerford, J. D. Lopez, and G. Hernandez. 2006b. Mating incidence of feral *Heliothis virescens* (Lepidoptera: Noctuidae) males confined with laboratory-reared females. J. Cotton Sci. 10: 105–113.

Blanco, C. A., O. P. Perera, D. Boykin, C. Abel, J. Gore, S. R. Matten, J. C. Ramírez-Sagahon, and A. P. Terán-Vargas. 2007a. Monitoring *Bacillus thuringiensis*-susceptibility in insect pests that occur in large geographies: how to get the best information when two countries are involved. J. Invertebr. Pathol. 95: 201–207.

Blanco, C. A., A. P. Terán-Vargas, J. D. Lopez, Jr., J. V. Kauffman, and X. Wei. 2007b. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. Fla. Entomol. 90: 742–750.

Gahan, L. J., F. Gould, and D. G. Heckel. 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens.* Science (Wash., D.C.) 293: 857–860.

Gould, F., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545–1559.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. G. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens.* Proc. Natl. Acad. Sci. U.S.A. 94: 3519–3523.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

Gould, F., and B. Tabashnik. 1998. Bt-cotton resistance management, pp. 67–105. *In* Mellon and J. Rissler (eds.) Now or Never: serious new plans to save a natural pest control. Union of Concerned Scientists, Cambridge, MA.

Hardee, D. D., L. C. Adams, and G. W. Elzen. 2001. Monitoring for changes in tolerance and resistance to insecticides in bollworm / tobacco budworm in Mississippi, 1996–1999. Southwestern Entomol. 26: 365–372.

Heckel, D. G., L. C. Gahan, F. Gould, and A. Anderson. 1997. Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 90: 75–86.

Huang, F., B. R. Leonard, and D. A. Andow. 2007. Sugarcane borer (Lepidoptera: Crambidae) resistance to transgenic *Bacillus thuringiensis* maize. J. Econ. Entomol. 100: 164–171.

Luttrell, R. G., R. T. Roush, A. Ali, J. S. Mink, M. R. Reid, and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *H. virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985–989.

Mahon, R. J., K. M. Olsen, S. Downes, and S. Addison. 2007. Frequency of alleles conferring resistance to the Bt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 100: 1844–1853.

Matten, S. R., and A. Reynolds. 2003. Current resistance management requirements for Bt cotton in the United States. J. New Seeds 5: 1317–178.

Parajulee, M. N., D. R. Rummel, M. D. Arnold, and S. C. Carroll. 2004. Long-term seasonal abundance patterns of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in the Texas High Plains. J. Econ. Entomol. 97: 668–677.

Roush, R. T. 1997a. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pestic. Sci. 51: 328–334.

Roush, R. T. 1997b. Managing resistance to transgenic crops, pp. 271–294. *In* N. Carozzi and M. Koziel [eds.], Advances in insect control: the role of transgenic plants. Taylor & Francis, London, United Kingdom.

SAS Institute. 2001. SAS, version 9.1. SAS Institute, Cary, NC.

Shaver, T. N., and J. R. Raulston. 1971. A soybean-wheat germ diet for rearing tobacco budworm. A. Entomol. Soc. Am. 64: 1077–1079.

Siegfried, B. D., T. Spencer, and J. Nearman. 2000. Baseline susceptibility of the corn earworm (Lepidoptera: Noctuidae) to the Cry1Ab toxin from *Bacillus thuringiensis.* J. Econ. Entomol. 93: 1265–1268.

Sparks, T. C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. Entomol. Soc. Am. 27: 186–192.

Stodola, T. J., and D. A. Andow. 2004. F_2 screen variations and associated statistics. J. Econ. Entomol. 97: 1756–1764.

Terán-Vargas, A. P., J. C. Rodriguez, C. A. Blanco, J. L. Martinez Carrillo, J. Cibrian Tovar, H. Sanchez Arroyo, L. A. Rodríguez del Bosque, and D. Stanley. 2005. Bollgard cotton and resistance of the tobacco budworm (Lepidoptera: Noctuidae) to conventional insecticides in southern Tamaulipas, Mexico. J. Econ. Entomol. 98: 2203–2209.

Received 14 January 2008; accepted 13 March 2008.